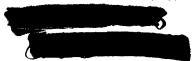
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Protection Branch Report of Test No. 20-63 N65 16837

THE EFFECT OF NICKEL-CADMIUM BATTERIES UPON BACTERIAL SPORES

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Prepared by:

Approved by:

DOROTHY M. PORTNER
Decontamination Section

ROBERT K. HOFFMAN

Chief, Decontamination Section

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Chief, Protection Branch

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Chief, Physical Defense Division

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Protection Branch Report of Test No. 20-63

The Effect of Nickel-Cadmium Batteries Upon Bacterial Spores

INTRODUCTION

In line with the problem of developing sterilization procedures for interplanetary spacecraft, it has been demonstrated that the nickel-cadmium battery cannot withstand the necessary temperature or radiation exposure required for sterilization with these techniques. It has been suggested, however, that the battery is self-sterilizing because of the high hydroxide content and therefore would not require special sterilizing treatment. In order to test this hypothesis Goddard Space Flight Center submitted several batteries to Fort Detrick for evaluation.

MATERIALS AND METHODS

The nickel-cadmium battery contains an electrolyte which is approximately 30 per cent potassium hydroxide, a separator material of polypropylene felt, and the grid or support for both the positive and negative plates of powdered nickel metal sintered to form a sheet containing 80 per cent voids. These voids are subsequently filled with cadmium hydroxide (the negative) and nickelous hydroxide (the positive). Charging changes the former to cadmium metal and the latter to nickelic hydroxide.

The test procedure, in general, consisted of determining whether spores of <u>Bacillus subtilis</u> var <u>niger</u> would survive in the presence of a slice (about 1/8 inch thick) of the battery. Various dilutents, distilled water, 2 per cent hydrochloric acid and 0.5% sodium thioglycollate, were used as the medium in which the slice of battery was placed for assay. After various time periods, serial decimal dilutions in distilled water were made from the diluent containing a slice of battery. All bacteriological assays were made by the pour plate method using tryptose and/or thioglycollate agars for culture media. After an incubation period from 48 to 96 hours at 37° C, the ensuing colonies were counted.



RESULTS AND DISCUSSION

The results of four tests are given in Tables I. through IV.

In the first test, a slice of nickel-cadmium battery and <u>B. subtilis</u> var <u>niger</u> spores were put into each of two diluents, hydrochloric acid and distilled water, to determine whether the spore recovery obtained in the presence of the battery was affected by the pH of the diluent. Serial decimal dilutions of each 50 ml sample were made, but only dilutions 1:500 through 1:500,000 were plated since these dilutions would be sufficient to indicate whether or not spores would survive. The results indicated that the spore recovery was approximately the same over a 24 hour period for both diluents (Table I.). It, thus, appeared that if many spores had been present in the nickel-cadmium battery they could have been recovered in either diluent. There appeared to be no necessity to neutralize the potassium hydroxide within the battery before assaying for viable spores.

The second test was designed to test whether spores could be recovered if the nickel-cadmium battery was deliberately contaminated. The results clearly indicated that bacterial growth was inhibited (Table II.).

Test III was done to determine whether the observed inhibition was due to the metals in the battery that diffused into the diluent and were subsequently carried over into the agar plate. Thioglycollate agar, which is known to neutralize the inhibitory effects produced by some metals, and tryptose agar, the medium used in previous tests, were used in the assay of the slice of battery deliberately contaminated with spores. The results show that the inhibitory effects were partially overcome by the use of thioglycollate agar; moreover, a sporicidal effect seemed to be present (Table III.).

The last test was designed to determine whether spores would survive in a nickel-cadmium battery. Four slices of battery were deliberately contaminated with spores and one slice assayed immediately (15 seconds) and the other three after the spores were in contact with the battery for 1/2, 3 and 24 hours. Sodium thioglycollate solution instead of water was used as the assay diluent and only thioglycollate agar was used for culture medium. By this procedure, the inhibitory effects of the battery material carried over into the agar at the low dilutions was minimized and growth ensued. The results given in Table IV stronly suggest that microorganisms would not survive more than an hour or two in a nickel-cadmium battery. The spores that were recovered after 3 and 24 hours exposure were probably on the fibers protruding from the surface of the battery slice and not in direct contact with the alkali as would be expected with organisms naturally entrapped.

It is believed therefore that one type the land the tery tested is self-sterilizing.

Table I.

Recovery of Bacterial Spores in the Presence of Nickel-Cadmium Battery Slice in Hydrochloric Acid and in Distilled Water

| Contact Time | Hydrochloric Acid | | Distilled Water | | |
|-----------------|-------------------|----------------|-----------------|----------------|--|
| (hours) | pH | Spores/sample* | Hq | Spores/sample* | |
| o | 0.8 | | 7.6 | | |
| 3 | 2.2 | 13,900,000 | 12.0 | 26,500,000 | |
| 6 | 4.4 | 13,000,000 | 11.8 | 28,800,000 | |
| 24 | 6.0 | 10,100,000 | 11.8 | 24,200,000 | |

^{*} Inoculum 20,000,000 to 30,000,000

Table II.

Recovery of Bacterial Spores 1/2 after Direct Contact with the Nickel-Cadmium Battery Slice for 24 Hours

| Dilutions | Colonies/plate | pH of agar in plate $\frac{2}{}$ |
|-----------|----------------|----------------------------------|
| 1:10 | 000 | 8.4 |
| 1:50 | 0-0-0-0 | 7.4 |
| 1:500 | 2-0-0-0 | 7.1 |

Note: The agar surface of all sterile plates were subsequently inoculated with 10⁷ B. subtilis var niger spores. None of the plates at the 1:10 dilutions supported growth, half of the plates at 1:50 dilution supported growth, and all the plates at 1:500 dilution supported growth.

^{1/} 10,000 spores of B. subtilis var niger were placed directly on the battery.

^{2/} pH of sterile control agar was 7.1.

Table III.

Comparison of Two Agars for the Recovery of Bacterial Spores after Direct Contact for 15 Seconds and 24 Hours with Nickel-Cadmium Battery Slice

| | Av/Sample_ | 36,500,000 | 45,200,000 | 750*** | 2,750*** |
|-----------|------------|------------|------------|--------|----------|
| Dilutions | 1:500T | 81–65 | 87–94 | 9-0 | 0-0 |
| | 1:50T | INTC | TNTC | 0-0 | 9-0 |
| | 1,5T | TNTC | TNTC | 0-0 | 0-0 |
| | 1:500 | TNTC | TNTC | 1-2 | 4-7 |
| | 1:50 | 9-0 | TNTC | 4-3 | 14-14 |
| | 1:10 | 9-0 | 90 | 90 | 90 |
| | Agar | Try | Thio | Try | Thio |
| | PH. | 13.1 | | 13.4 | |
| Contact | Time | 15 sec | | 24 hrs | |

Try = tryptose agar

Thio = thioglycollate agar

= thousand

TNTC = too numerous to count

* = diluent with battery slice

** = growth only on 1/2 plate

= based on the counts from the 1:500 dilutions since some inhibitory effects seem to exist at the 1:50 dilution. ***

Table IV.

Recovery of Bacterial Spores after Direct Contact with the Nickel-Cadmium Battery Slice for Various Time Periods

| Contact Time (hours) | pH of Sample | Av. Count/Sample |
|----------------------|-----------------|------------------|
| Initial | 13.0 | 51,400,000 |
| 1/2 | 13.0 | 16,400 |
| 3 | 13.0 | 13 |
| 24 | 13.0 | 8 |

Note: pH of the agar was affected at the lower dilutions, that is, pH 8.0 at 1:10 dilution, pH 7.4 at 1:50 dilution, pH 7.1 at 1:500 dilution and pH 7.0 for all subsequent dilutions.